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A STUDY OF PLASTIDS AND MITOCHONDRIA IN PRESSIA AND CORN

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In this paper certain observations upon plastids and mitochondria are recorded. In my opinion it would be premature as yet to formulate any conclusions as to the fundamental significance of these bodies, though the preparations I have obtained are clean-cut and definite.

In order to make clear the position assumed in regard to the structures in question, it may be well to state, at the outset, that the term plastid will be used to include not only the leuco-, chloro-, and chromoplasts, but also the *Anlagen* for the same. The name mitochondria, on the other hand, will be restricted to those granules which are not, in general, preserved by the usual methods of fixation—those which, in other words, are dissolved in acetic acid or in alcohol and are fixed by the use of osmic acid, formalin, etc. The mitochondria, moreover, color more or less specifically with various stains. I shall in general use Benda's term *mitochondria*, rather than others that have been proposed, though the etymology of the word implies a thread-like form not always present.

In cells prepared by what are known as the mitochondrial methods, these bodies, by reason of their number and intense affinity for the dyes become in many cases quite the most striking features of the protoplast. The only reason that they were neglected by cytologists for so long a time is the fact that they are dissolved by the processes commonly used to demonstrate nuclear phenomena.

Interest in the granular constituents of the cytoplasm has greatly increased in the last few years, though the idea of their importance is not a new one. To Altmann, in 1886, is due the formulation of what is generally known as the granular theory of protoplasmic structure. Hanstein, in 1882, had maintained that protoplasm is made up of minute granules, which he termed microsomes, and a homogeneous fluid in which the microsomes float. Altmann, using a special technique, consisting essentially of fixation with osmic acid and potassium dichromate, was able to demonstrate the presence in various tissue cells, as well as in the chromosomes, of numerous granules to which he gave the name of *bioblasts*. These bodies he regarded as possessing an independent existence, and to them he imputed the power of growth and of multiplication by division. He also believed that they

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are transformed into the products of secretion such as fats, glycogen, pigments, etc., in the cells. But Altmann, besides claiming for his bioplasts the powers already noted, which are to a certain extent the same as those believed by modern exponents of the mitochondrial theory to reside in the mitochondria, held that the bioplasts are the morphological units of living matter, constituting the essential elements of protoplasm. The difference, in short, between Altmann's hypothesis and the more modern theories of the mitochondria lies in the fact that the bioplasts were postulated to possess an independence and autonomy, to quote Regaud, which the mitochondria as cell organs are not thought to exhibit.

Benda, in 1898, having devised a more specific and definite method of fixation and staining, may be regarded as the founder of the modern mitochondrial theory. He introduced the terms *mitochondria* and *Chondriomiten*, from *μίτος* a thread, and *χόνδριον* a grain. *Chondriosome* and *chondriocont*, later introduced by Meves, have also come into use, the former being used synonymously with mitochondria, and the latter being applied by Meves to homogeneous threads. The collective term *chondriome* is also often employed.

Regaud ('11) suggests the possibility that the mitochondria "fix" and "elaborate" the substances necessary for the functioning of the muscle cells, such as glycogen, and that they also perform a similar function in the case of the gland cells, such as the secretory cells of the kidney and of the salivary glands. He says: "*Les mitochondries sont les organites sur lesquels se fixent les substances destinées au fonctionnement chimique de la cellule; ces organites concentrent les substances fixées, les élaborent et les transforment en produits de sécrétion, auxquels ils servent même de supports, dont ils sont les plastes.*" The mitochondria are "*les agents de l'intussusception élective, c'est-à-dire, de l'introduction dans la cellule des substances amenées par le sang.*"

Dubreuil ('13) believes that the mitochondria are responsible for the production of fat in the cells, through a process of differentiation. According to Dubreuil, a lipid vesicle is first formed, this process being followed by the development of a fat droplet. The diagrams to illustrate this process, showing the mitochondria becoming vesicular and forming both hollow spheres and "hand-mirror-like" forms, exhibit a remarkable similarity to the series of changes which Guilliermond and others show in their illustrations of the production of plastids by the mitochondria in plant cells.

Van der Stricht ('09) has presented some very suggestive studies upon the genesis of yolk-spheres in the egg. He finds that the mitochondria are, at first, confined to a region around the nucleus from which they migrate outward and are gradually transformed into yolk-spheres.

Lewis and Lewis ('15) have employed a novel method for the study of mitochondria with most interesting and suggestive results. Portions of the living embryo of the chick were segregated under aseptic conditions and cultivated in Locke's solution, in hanging drop cultures. In such cultures

the mitochondria may be studied in the living, growing condition, fixed while under observation and thus preserved as permanent preparations. Experimental methods may be employed, by which the effect of different stains and reagents may be observed directly. Such preparations, placed in the electric incubator at a temperature of 39° to 40° C., show a beginning of growth in from 10 to 20 hours. The new growth is attached to the cover glass and is several cell layers in thickness at first, thinning out to a single cell layer around the edges. Although conditions are necessarily somewhat different from those of the normal somatic environment, especially as regards circulation, and in the limited supply of oxygen since the cultures must be hermetically sealed, the processes of growth and of mitosis seem to go on as usual for a period of about three days. After this the process slows down, growth ceases, and the cells finally die.

Mitochondria, conforming to the usual criteria, were found to be present in all cells studied. Osmic acid vapor proved to be the best fixative, while the vapors of acetic and other acids caused immediate and total disintegration of the mitochondria. The bodies appeared as threads and granules of the most varied shapes and sizes, just as they have so often been described in fixed preparations. They are not, however, constant in form or in size under these conditions, but are constantly changing in appearance. They are described as undergoing division, as fusing to form larger bodies, and as disappearing and reappearing in the cells in a manner not accounted for. During mitosis they are distributed regularly about the cell, so that they are apportioned to the daughter cells in approximately equal numbers. No connection of the mitochondria with the production of fats, such as described by Dubreuil ('13), was noted in these preparations. In conclusion, the authors state: "The mitochondria are extremely variable bodies, which are continually moving and changing shape in the cytoplasm. They appear to arise in the cytoplasm and to be used up by cellular activity. They are, in all probability, bodies connected with the metabolic activity of the cell."

Lewis and Robertson ('16) found that the above described method of tissue culture was well adapted to the study of spermatogenesis in the grasshopper, *Chorthippus curtipennis*. In the young spermatid the mitochondria were in the form of a granular *Nebenkern*. After certain internal changes, the import of which was not clear, the *Nebenkern* was seen to divide into half-spheres. These half-spheres then elongated to form granular sacs, which, as the tail grew out, formed two irregular strands. These irregular strands finally fused to form "two continuous threads of even width, extending from the centrosome body, or middle piece, almost to the end of the tail."

The authors conclude that "it does not seem possible that bodies which have to do only with the metabolic activities of the cell should undergo such an exact behavior as shown, for instance, by the division of the *Nebenkern*

into two equal parts and the development of these two sacs of mitochondria into two long threads of mitochondria in the spermatozoon."

There has been a tendency, especially among the earlier observers of mitochondria in plant cells, to ascribe to these bodies a nuclear origin. A perusal of the literature clearly indicates that the work of Goldschmidt ('04) is largely responsible for this, though the inception of the chromidial hypothesis doubtless owes its origin, as Dobell ('09) states, to the work of R. Hertwig, supplemented by that of Schaudinn, dealing with the occurrence of such bodies in the protozoa.

Meves ('04), working with the tapetal cells in the anthers of *Nymphaea alba*, is credited with having made the first observations of mitochondria in plant cells. Meves shows two very clear-cut and beautifully drawn figures, of which his description is as follows: "*Enthält sie lange, unregelmässig gewundene, ziemlich dicke Fäden, welche sich mit Eisenhämatoxylin intensiv schwarz gefärbt haben. Diese Fäden können nicht wohl etwas anderes sein, als die von tierischen Zellen bekannten Chondromiten.*"

It is not so much the question of origin, whether *sui generis* or chromidial, however, that has engaged the attention of later workers upon plant mitochondria, as that of their relation to other structures in the cell and of their universality. Lewitsky and Pensa, working independently, have advanced a contention which promises to furnish material for controversy for some time to come.

Lewitsky ('10) studies the root-tip and stem-tip of *Asparagus officinalis* treated according to the Benda method and stained with both the Benda stain and haematoxylin. He finds mitochondria corresponding to those described in animal cells, both in general appearance and in staining reaction, with no evidence whatever of a nuclear origin. The mitochondria appear short and rod-shaped, in the dermatogen; somewhat larger and with a tendency to swell at the ends, in the second layer; still larger in the third layer, while deeper in the assimilative tissue "dumb-bell" forms are seen, "similar to those well known in division figures of the chloroplasts." Next, still larger bodies are shown which appear as if they have come from the separation of the two halves of the "dumb-bells." These are followed by figures of the young chloroplasts, and finally by the mature bodies. Meanwhile, the earlier forms of the mitochondria, combined with the "division figures" of the intermediate regions, lead him to conclude that the mitochondria are the *Anlagen* of the chloroplasts. Upon fixing some of the same material in alcohol and acetic acid, Lewitsky found that the mitochondria were no longer to be seen in the cells, while the chloroplasts appeared as usual. This is taken as evidence of a chemical as well as a morphological transformation of the mitochondria, in producing the chloroplasts.

Guilliermond ('11, '13, '14) has published a number of papers in which he describes the mitochondria in all sorts of plants, his purpose being, on

the whole, not so much to demonstrate the presence of mitochondria therein as to substantiate his theory of their functional rôle. In addition to portraying the same processes of development as those described by his predecessors, Guilliermond seeks to show that the mitochondria of plant cells possess the same "elaborative" functions that have been postulated for animal mitochondria by Regaud and others. In a resumé of the work upon mitochondria, published in "*La Revue générale de Botanique*" in 1914, he says: "*Ces recherches démontrent surabondamment que les mitochondries sont des plastes, c'est-à-dire des organites qui élaborent les produits de sécrétion. . . . A la suite de ces recherches, la cellule apparaît désormais avec un nouvel élément: le chondriome, dont la présence est aussi constante et joue un rôle aussi essentiel que le noyau. . . . La découverte des mitochondries transforme donc la cytologie.*" Mottier ('18) has discussed the literature on the relations of chondriosomes and plastids and it need not be further summarized here.

OBSERVATIONS

Although I was particularly concerned, in my own investigations, with obtaining evidence as to the relationship between mitochondria and plastids, the mitochondrial methods of fixing and staining were tested first upon animal tissue. A number of preparations were made from the testes of the grasshopper, *Caloptenus femurrubrum*. They were fixed with Benda's solution, with Bensley's, and with Flemming's strong solution, and stained in various ways, in order to compare the results obtained with different combinations.

Benda's method of fixing and staining gave by far the best results, the mitochondria being well differentiated and shown in the characteristic changes through which they pass, as described by Lewis and Robertson ('16) in their observations upon the behavior of the mitochondria during spermatogenesis in the grasshopper, *Chorthippus curtipennis*. In my preparations, Bensley's method gave unsatisfactory results in general protoplasmic differentiation, though the mitochondria were well preserved and stained.

Benda's process, according to the formula given below, was therefore used in most of my plant material.

- Fixation. I. Benda's Flemming, 8 days.
 (1 percent chromic acid, 15 cc.,
 2 percent osmic acid, 4 cc.,
 3 drops acetic acid.)
 II. Wash in water, 1 hr.
 III. Pyroligneous acid (rectified) and chromic acid 1 percent, equal parts, 24 hrs.
 IV. Bichromate of potassium, 2 percent, 24 hrs.
 V. Wash in water, 24 hrs.
 VI. Dehydrate and imbed. Cut 5 microns thick.

- Staining. VII. Iron alum, 4 percent, 24 hrs.
 VIII. Rinse in distilled water.
 IX. Alizarine sodium sulphonate, 24 hrs.
 (1 to several cc., saturated alcoholic solution, in 80 to 100 cc. distilled water.)
 X. Heat in crystal violet, 3 to 5 min. after vapor rises.
 (3 percent alcoholic solution crystal violet and aniline water, equal parts.)
 XI. Rinse in distilled water.
 XII. Destain in 15 percent acetic acid.
 XIII. Wash in running water, 5 to 10 min.
 XIV. Dry with filter paper. Dip in absolute alcohol.
 XV. Bergamot oil, followed by xylol.

This stain gave a very beautiful result, the mitochondria being colored a dark violet or blue, with a background of old rose. Only in exceptional cases was the background too light to be satisfactory.

Regaud's method of fixing and staining was also employed, to some extent, upon the plant tissues.

- Fixation. I. Bichromate of potassium, 3 percent, 80 vols., and commercial formalin, 20 vols., four days.
 II. Bichromate of potassium, 3 percent, eight days.
 III. Wash in water, 12 hrs.
 IV. Dehydrate, imbed, and cut 5 microns thick.
 Staining. V. Stain with iron-alum-haematoxylin (Heidenhain's method).

This is the formula as given by Guilliermond, who has used it in much of his work. In my preparations it gave good results, at times, while again the results might be very bad, possibly due to impurities in the formalin. The Benda fixation does just as well as a preparation for the iron-alum-haematoxylin as for the crystal violet-alizarin stain, this combination being often used.

CORN

In the root-tips of corn, of the "Canadian Early, Yellow Flint" variety, fixed according to Regaud's formalin-bichromate method, the time being shortened to four hours in the fixative (I), and eight hours in the bichromate (II), the cytoplasm in the embryonic region appears gray and filled with exceedingly numerous jet-black mitochondria, when stained with iron-alum-haematoxylin. In this region the mitochondria are globular, ellipsoid, or short rod-shaped. In the root-cap, next to the tip, they are similar, gradually lengthening from short rod-shaped to elongated, filamentous forms as one passes from the embryonic region toward the periphery of the cap.

In the root-tip proper, passing back into the region of elongated cells in

the plerome, a marked change occurs. The mitochondria now appear as elongated, thread-like bodies, seeming to have arisen from the spherical and ovoid forms by a process of lengthening and thinning. Of these elongated forms, many appear hooked, or in some cases vacuolate, at the ends. Mingled with these thread-like structures are others which appear circular, as if a thread had formed a ring, or possibly a globular form had changed in appearance so that it resembles a hollow sphere. At times, chains of small granules are seen, probably due to the breaking up of a filament. Figure 1 shows the large number and greatly varied shapes of the mitochondria in this region.

Passing outward from the region of elongated cells in the plerome and entering the periblem, the cells are found crowded with mitochondria, mostly spherical in shape. It appears as if the mitochondria do not, in general, elongate in this region, though they increase in size, approximately to the same extent in all directions. As will be shown later, these enlarged bodies of the periblem are in reality not mitochondria, but plastids, though they stain in exactly the same manner with the haematoxylin. Whether there are any plastids present in the plerome region as well, I am not as yet prepared to say. Figure 2 shows a cell from the periblem in mitosis, drawn to the same scale as figure 1, namely, nine hundred diameters. Figure 3 shows a similar cell enlarged to twice the size.

As may be seen from these drawings, the mitotic figure is shown very much as it appears in the usual method of fixation, with the exceptions to be noted. The spindle fibers are but faintly shown, if visible at all, although the general outline of the spindle appears as it normally does. The chromosomes are rather attenuated, though they sometimes show a shadowy outline of surrounding material, as if only the central part of their structure had been stained. The difference between this method of fixation and those methods designed primarily for showing nuclear structure, is much more marked in the resting cell. Here the nucleus appears, in general, more uniformly granular and less reticulate than it does in preparations fixed, say, in Flemming's strong fluid. The nucleole also is different, appearing much larger than we are accustomed to see it in fixed material. In short, the mitochondrial methods of fixation do not seem to alter the appearance of the protoplast so much as do the usual types of fixation, since with the mitochondrial methods the structure appears very much as it is described by Lewis and Lewis ('15) and by Lewis and Robertson ('16), in their observations upon living tissue cells.

The root-tips from which the above described preparations were made were grown in the laboratory during the coldest part of the winter and not under constant temperature conditions. To this I attribute the difference between the granular content of the cytoplasm in this material and in the preparations next to be described.

Root-tips of the same variety of corn, grown later in the season and under

more favorable conditions of temperature and light, prepared and stained by the Benda method, show a much greater proportion of the enlarged vesicular bodies which stain like mitochondria. In these preparations, however, there is very good evidence that these bodies are in reality plastids, since they show a lighter colored internal portion, evidently consisting of starch. In regions apart from the meristem these bodies are very abundant. In the plerome (fig. 4) the filamentous mitochondria often appear swollen at the ends or sometimes in other portions, while the plastids, ovoid or spherical in shape, may contain from one to several starch grains each as shown in figure 5.

In the periblem, on the other hand, there are in the intermediate regions of the tip, ovoid, spherical, or irregular masses of a much more solid appearance, in general, but often showing a number of discrete spherical granules in their interior. Numerous smaller spherical or ovoid bodies are also present, scattered about through the cytoplasm, which are dark blue in color, taking the stain exactly as do the plastids and the filamentous mitochondria.

These preparations, stained and fixed according to the Benda method, as previously stated, show the nucleus finely granular in appearance and of an old-rose color, as dark, relatively, as shown in the drawings, figures 4 and 5. The nucleole is apparently of a denser consistency and stains a darker shade of the same color. The cytoplasm is very well preserved and stains somewhat lighter than the nuclear material.

A slide of the above described material was freed from paraffin with xylol, bleached for a few minutes in hydrogen peroxide, and treated with potassium iodide-iodine solution, with the object of testing for starch. Under this treatment the bodies which have been referred to as plastids show a bluish color in their interior, but hardly pronounced enough to be considered a convincing demonstration of the presence of starch. A subsequent treatment of the slide with a solution of iodine in chloral hydrate, however, gave better results, differentiating the bodies so that they appear as plastids with included starch grains, as indicated by the blue color of the interior.

Next, a similar slide from the Benda fixation was stained with the Flemming three-color process, the red being left decidedly strong. The mitochondria, both granular and filamentous, are now strongly stained by the safranin, while the plastids are colored blue. In the intermediate regions of the tip, in the periblem area, the plastids are rather lightly stained, and within each of them there are a varying number of spherical granules which are stained by the safranin in the same manner as the mitochondria, except that they are, generally, a brighter red. In figure 6, *a*, *b*, *c*, *d*, and *e*, a number of these plastids are shown with their included red-staining granules. As one leaves the intermediate regions of the tip and proceeds toward the proximal portion, the red bodies within the plastids gradually lose their

sharply defined appearance and bright color, becoming gradually dimmer. Still farther back in the tip the red color disappears entirely, the plastids appearing more or less opaque or more uniformly blue in color, as shown in figure 6, *g*, *h*, and *i*.

It has not been possible to determine, so far, how this association of the bright red granules with the light blue plastids comes about, though it might be imagined, from observations I have made, such as the appearance of two darkly staining red granules at the ends of a light blue ellipsoid, that the mitochondria are surrounded by, or become surrounded by, a substance which makes up the body of the plastid; that they divide within this plastid substance and afterwards produce the starch grains within it, or themselves become changed into starch. This appearance suggests a relation to starch formation similar to that of the pyrenoid, as described by McAllister ('14).

In other cases, however, bodies were observed which are made up of a dark red peripheral layer surrounding a light blue center. Both the latter structures and the ellipsoids occur in the intermediate regions of the periblem, between the red-staining mitochondria and the blue plastids with their red, granular inclusions. I wish to emphasize the fact that both the mitochondria and the plastids are exceedingly numerous in the regions indicated and that the staining reactions and the differentiation of the bodies described as occurring in the plastids are very definite. In many cells of the periblem, in the intermediate region of the tip, the nucleus is practically surrounded by a number of large plastids containing red-staining granules, while the cells nearer the tip are crowded with mitochondria which also color strongly with the safranin. Nevertheless, while the existence of these bodies is clearly demonstrable, I do not wish to imply that the seriation to prove their inter-relations is equally evident.

PREISSIA COMMUTATA

The situation in connection with the cytoplasmic inclusions of the liverworts and of the Bryophyta in general, appears to be in special need of investigation, not only on account of the fact that a perusal of the literature shows a considerable difference of opinion as to the real nature of the various bodies in question, but also on account of the very great interest attached to the group by virtue of its intermediate position in relation to the flowering plants on the one hand, and to the algae on the other.

While the interest centers mainly in the plastids and their genetic relations, the oil bodies of the liverworts have received considerable attention from investigators, with no very definite results as far as their real nature and origin is concerned. Pfeffer ('74) is credited with having made the first really fundamental and comprehensive study of the oil bodies. In his opinion they are formed by the aggregation of a large number of small oil droplets which are already visible in the very young cells. While he, at first, maintained that the bodies originate in the cell sap, he later

agreed that it might be possible that they come from the cytoplasm, but that they finally lie in the vacuole. He believed the principal constituent of the bodies to be a fatty oil, since their contents dissolve in alcohol, benzol, ether, etc. In addition to the fatty oil, some other material was found to be present, appearing as a residue after the solution of the oil. The membrane which he observed surrounding the bodies after they had been stained with iodine and with cochineal, was apparently composed of some protein material, insoluble in dilute acid and in alkalies. Since the bodies were unchanged after a three-months' cultivation of the plants in darkness, and since they were still present, in such cases, in the very young cells, just as in the plants which had grown in the light, he concluded that they have no significance in nutrition and that they are merely products of excretion.

Wakker ('88) included the oil bodies of the liverworts under the "elaioplasts," as he had named the oil-producing bodies which he had demonstrated in many phanerogams. Although these bodies appear, in life, to lie in the cell sap, Wakker showed by abnormal plasmolysis that they, in reality, lie in the cytoplasm. He believed them analogous to leucoplasts and chloroplasts, holding that they multiply by division and are distributed to the daughter cells in mitosis.

Von Küster ('94) believes that the oil bodies are formed from a protein "stroma" and that the apparent membrane seen in fixed material is an artefact. Since he was not able to see the membrane in living material, even with the strongest magnification, he considered it a precipitation membrane, formed by the interaction of the oil and the substance of the stroma. He showed that the membranes were not visible in material which had been fixed in osmic acid and stained with gentian violet. He also showed that a double membrane could be formed by the use of dilute alcohol, followed by strong. In regard to the nature of the bodies, he believed with Pfeffer that they are excretion products. He did not believe that the oil bodies undergo division and are handed down from cell to cell, but thought that they are newly formed in each cell.

Garjeanne ('03) believes that it is possible to show that the oil bodies are in reality merely vacuoles filled with oil which is secreted from their walls; that they lie in a half-fluid transition substance; that they increase by division, and that the membrane is an artefact. He admits, however, that the picric acid which he used in his demonstrations acts very rapidly, so that observations upon young cells must be made within one minute after the application of the acid, before disorganization of the cell contents sets in. He compares the oil bodies to the leucoplasts in their origin from *Anlagen*, which he believes to be vacuoles in the case of the leucoplasts also. After being fully formed, the oil bodies, he says, are no longer capable of division, remaining thereafter unchanged. In addition to the vacuoles, or *Anlagen*, of the oil bodies, he describes other minute structures which are

similar to the young stages of the oil bodies but which differ from them in their chemical properties. Since, in addition to the *Anlagen* of the oil bodies and to these structures which are similar to them, Garjeanne mentions also the *Anlagen* of the chloroplasts, it would seem that he believes that there are present in the cells of some liverworts, at least three varieties of specifically different granules.

Rivett ('18), in an account of certain observations upon *Alicularia scalaris*, finds that the results of staining or fixing the entire leaves with 2 percent osmic acid "confirm the view that the oil is secreted in vacuoles." This author also finds certain refractive granules present in the cells of both the growing point and the older leaves, which differ in their chemical reactions from the young oil bodies, apparently agreeing with the observations of Garjeanne in this respect. In the meristematic regions a "chondriome-like structure" was observed, but no evidence was found "that the chondriosomes were either transformed directly into plastids by a secretion within their own substance, or that they are the instigators of secretory action on the part of the protoplasm." No evidence was found that the refractive granules were chondriosomes, "since their appearance in the stained mature cells is quite different from that of the chondriome of the actively dividing cells."

As already noted, mitochondria have also been described in the liverworts by Scherrer ('13) and by Mottier, ('18), neither of whom was able to find any connection between these bodies and the chloroplasts. Scherrer made a special study of *Anthoceros*, a form which possesses the greatest interest since it has a "pyrenoid" in some of its chloroplasts, suggesting a close relationship with the algae in respect to its method of starch formation.

The pyrenoid of *Anthoceros* has been described by McAllister ('14), who finds that it consists of from 20 to 300 minute lenticular bodies, which lie near the center of the chloroplast and which stain bright red with safranin. McAllister states that there can be no doubt that these bodies are transformed directly into starch, since "there is a gradual change of the color reaction, from the brilliant red of the pyrenoid bodies to the blue of the starch grains." On the other hand, he says that, in the cells of the archesporium, in the spore mother cells, and in the assimilative cells of the sporophyte, starch is formed without the intermediary action of a pyrenoid—apparently arising *de novo* in the chloroplasts.

The observations of Davis ('99) agree with those of McAllister in this respect, since he states that the first clear indication of the chloroplast in the spore mother cells of *Anthoceros* is the sharp staining of the starch grains—purple with the gentian violet.

McAllister states, further, that there is no doubt that if the *Anlagen* of these bodies (the starch grains of the spore mother cells) are present in the plastids of the archesporial cells, they are too minute to be distinguished with the highest magnifications. This, however, does not necessarily follow,

since neither McAllister nor Davis reports having tried the mitochondrial methods of fixation.

Although *Anthoceros*, in general, has but one chloroplast in each cell, Campbell ('06) has described a species from Jamaica which has several chloroplasts—as many as eight in the cells of the inner tissue—so that the connection of *Anthoceros* with other liverworts, in this respect, is not so remote as might at first appear. All in all, *Anthoceros* is obviously a most interesting form and one upon which considerably more work is necessary.

Observations

Portions of the thallus of *Preissia commutata*, upon which the gamete-bearing discs were beginning to appear, were fixed according to Benda's formula, imbedded, and cut 5 microns in thickness. The smallest disc studied was about one millimeter in diameter. Figure 7 represents a portion of such a disc, as it appeared when stained with the Benda method, and with a magnification of three hundred eighty-four diameters. The darker cells in this section, three of which are shown in figure 7, present the same relatively dark appearance in the unstained, unbleached preparations. They are filled with a dense mass of thin-walled, spherical bodies which stain darkly with osmic acid as well as with the mitochondrial stains. Treatment with a preparation of alcannin shows the periphery of these cells made up of an alveolar substance, staining purplish gray, while the central portion contains a mass of material which stains a dark red. These central masses are the "oil bodies" of Pfeffer and others, or the "elaioplasts" of Wakker.

In this section there are also differentiated two other sorts of bodies. The smaller, more uniform variety, which may be seen occupying the periphery of the cells, is apparently of a fatty nature, since they are somewhat darkened in the unbleached cells. They appear granular and plastic, being flattened more or less along the cell walls. There appears to be no difference between the periphery and the interior of these bodies, since no bounding membrane nor any lighter-colored area in the interior can be made out in the stained slides. From the larger ones, two microns or more in diameter, of which there are usually a larger number of about the same dimensions in these cells, they seem to grade down to extremely minute granules.

The other bodies vary much more in size, there being no two of any one size in the cell. They include, doubtless, the bodies described as oil droplets by Pfeffer. Figures 8, 9, and 10 show a few of the cells taken from the same group as figure 7, but more highly magnified. The more uniformly colored bodies in these cells, mostly seen in side view around the periphery of the cell, belong to the first class, while the more rounded ones, with dark borders, belong to the second class. Of the latter, the larger ones may appear to be in a state of division or fusion, a number, of varying sizes, often

appearing in groups; figure 11, plate II, shows a peripheral cell from a young disc containing the two sorts of bodies of the second class.

Returning to figure 7, as one proceeds from the periphery of the young disc toward its center, the bodies of the second class appear, on the whole, smaller. There seems to be a gradual decrease in their size correlated with an increase in their number, up to a depth of about two cell layers below the areolae. Here, bodies of this class begin to decrease in number, indicating a diminution in the amount of oil in the cells, while bodies containing from one to several starch grains begin to be seen: figures 8 and 9.

This seriation, suggesting that at least some of the bodies are plastids, is better shown in figures 13 and 14, taken together, which were drawn from a somewhat older disc than that from which figure 7 was taken. Figure 14 shows two cells from still deeper within the disc, as compared with figure 13, from a central lenticular area in which all the cells are of this type and packed with storage starch. These plastids, for such they appear to be, are stuffed with starch and now appear merely as enveloping films, enclosing and separating the starch grains. The character of these starch grains, which show a definite hilum when stained in certain ways, as well as their general appearance and distribution, would indicate that they are, as already suggested, storage starch and that the grains are very likely stratified. It was also found on staining such a section as that shown in figure 12 with iodine, that the starch reaction was given by all the bodies of this character, even to some of the smaller ones in the peripheral layer of the disc. Figure 15 represents a series of stages in outline as they are seen in the development of starch in this disc.

In the chloroplasts of the thallus, starch is also present in large quantities, and toward the interior of the thallus there is a region in which the cells are moderately full of swollen plastids, each containing a number of plump starch grains, not at all lenticular in appearance as they are so often figured. Lenticular-shaped starch grains are found, however, in the chloroplasts of cells at and near the periphery, especially in the vicinity of the growing point of the thallus. In the cells of the disc which contain starch, as well as in those of the thallus, the smaller, more plastic, and darker-staining bodies are still seen, arranged around the periphery. The largest of these should be the young chloroplasts.

In the apical cell of the thallus and in its immediate vicinity, bodies may be seen which I take to be the same as those already described from the peripheral cells of the young disc, though they are much smaller in size. In this region, also, and especially in the filamentous growths therefrom, mitochondria of various shapes appear, very much as described by Mottier ('18) for *Marchantia*.

DISCUSSION

It was at one time the accepted belief among botanists that chloroplasts arise *de novo* in the cytoplasm. This is definitely stated to be the case by

Sachs, in his Text-book of Botany, in 1882, where the process is compared to that of so-called free-cell formation. Since the work of Schimper, afterwards confirmed by that of A. Meyer, the opinion has become general that the three kinds of plastids found in plant cells, namely, leuco-, chloro-, and chromoplasts, are derived from minute, undifferentiated plastids which are *sui generis* structures of the cytoplasm. These chromatophores, as they are often called, were described, when seen in the living cell, as small, colorless, highly refractive bodies, recognizable in the egg and also in the embryonic cells. In older cells they have been said to retain the same appearance in some cases, while in others they become differentiated into leuco-, chloro-, and chromoplasts.

Schimper and Meyer believed that the undifferentiated plastids multiply by division and are handed down from generation to generation—that they have an individual existence in the cells. Considerable difficulty, however, was encountered by them in their attempts to demonstrate the presence of the plastids in the egg, owing to the fact that they were not easily seen in the living cells, and, as was admitted, they were difficult to stain at that stage. As Guilliermond expresses it, “that part of their theory remained very hypothetical.”

When the mitochondria were demonstrated, by means of a special technique, their study was first taken up by the zoologists, as has been shown, and special functions in the cell metabolism were imputed to them by Meves and others. Pensa is credited with having made the first observations tending to show that the mitochondria of plants may, possibly, be transformed plastids. This idea, developed by Lewitsky and Pensa and supported by numerous observations which have already been noted, was taken up by Guilliermond, who has attempted particularly to harmonize the functions of the mitochondria of plant cells with the theories concerning those of the mitochondria of animal cells as postulated by Meves, Duesberg, Regaud, Dubreuil, and others. He has confirmed the observations of Lewitsky and of Pensa by work upon a number of plants, including the seedling of barley. Here the mitochondria, followed from the meristem toward the green tip of the plumule, are shown as filamentous at first, followed by shorter and thicker forms which are sometimes dumb-bell shaped. From the appearance in succeeding cells of bodies which have the appearance of the separated halves of the “dumb-bells,” he believes that the latter divide. These bodies are followed by more rounded forms with a light center and a darker border. Finally, in the tip of the plumule, the mature chloroplasts are seen. While this series is considered by Guilliermond a very convincing proof of the mitochondrial origin of the chloroplasts, it is open to the objection that there seems to be no way of demonstrating that the *Anlagen* of the plastids are actually mitochondria and not merely young plastids.

On the other hand, the attempts of Rudolph, Sapêhin, Mottier, and

others to substantiate their contention that the *Anlagen* of the plastids are different from the mitochondria fail for the same reason—they are unable to demonstrate such a difference. Guilliermond ('14) claims that he is, in reality, upholding the Schimper-Meyer theory in that he is bridging a gap which the latter, with their cruder technique, were unable to fill. Schmidt ('12) also maintains that the work of Guilliermond and others confirms the Schimper-Meyer theory, but for a very different reason: they have simply been demonstrating the earlier stages of the plastids, according to Schmidt.

Lewitsky, however, claims to have shown that there are no other *Anlagen* of the chloroplasts than the mitochondria. His findings, with respect to *Asparagus officinalis*, which have already been referred to, are specifically as follows: a stem-tip of *Asparagus officinalis* was fixed with alcohol, 3 parts, and acetic acid, 1 part. This was stained with iron-alum-haematoxylin and light green. In the third and fourth cell-layers from above, where, in the preparations fixed by the Benda method, the somewhat large "chromatophore-dumb-bells" were found, these were no longer to be seen; only the usual "*Plasmagerüst*" was present. Since the mitochondria are destroyed by acetic acid and alcohol, and since all of the *Anlagen*, including the dumb-bell forms, disappear with the mitochondria under fixation with a combination of the above named reagents, these facts are taken as conclusive evidence of the identity of the mitochondria with the *Anlagen* of the plastids.

CONCLUSIONS

My own observations may be briefly summarized as follows:

1. As to size, an unbroken series of bodies, from mitochondria to plastids, can be traced in the root-tip cells of *Zea Mays* from the embryonic region backward. In *Preissia* this seriation is not so obvious.
2. The contention that such definitely staining bodies as the mitochondria exist and are normal constituents of the cytoplasm can hardly be questioned.
3. The evidence for the division of the mitochondria as well as that for their functions in heredity seems to me to be inadequate.
4. The further fundamental question as to the relation of the mitochondria to the remainder of the cytoplasm and the nature of the material in which they are imbedded, has not been cleared up.
5. Red-staining bodies are present in the plastids of corn, and, in some cases, in those of *Preissia* also.

BIBLIOGRAPHY

- Beer, R. 1905. On the development of the pollen grain and anther of some Onagraceae. *Beih. Bot. Centralbl.* 19: 286-311.
- Campbell, D. H. 1906. Multiple chromatophores in *Anthoceros*. *Annals of Botany* 20: 322.
- Cavers, F. 1914. Chondriosomes (mitochondria) and their significance. *New Phytol.* 13: 96-106; 170-180.
- Cowdry, N. H. 1917. A comparison of mitochondria in plant and animal cells. *Biol. Bull.* 33: 196-228.

- Davis, B. M.** 1899. The spore mother cell of *Anthoceros*. Bot. Gaz. **28**: 89-109.
- Derschau, M. v.** 1907. Über Analogieen pflanzlicher und tierischer Zellstrukturen. Beih. Bot. Centralbl. **22**: 167-190.
- . 1911. Ueber Kernbrücken und Kernsubstanz in pflanzlichen Zellen. Arch. Zellf. **7**: 424-446.
- Dobell, C. C.** 1909. Chromidia and the binuclearity hypothesis. Quart. Journ. Micr. Sci. **53**:
- Dubreuil, G.** 1913. Le chondriome et le dispositif de l'activité sécrétoire. Arch. Anat. Micros. **15**: 53-151.
- Duesberg, J.** 1910. Les chondriosomes des cellules embryonnaires du poulet et leur rôle dans la g n se des myofibrilles. Arch. Zellf. **4**: 602-671.
- Duesberg, J., and Hoven, H.** 1910. Observations sur la structure du protoplasme d s cellules v g tales. Anat. Anz. **36**: 96-100.
- Faur -Fremiet, E.** 1908. La structure des mati res vivantes. Bull. Soc. Zool. France **33**: 104-106.
- . 1910.  tude sur les mitochondries des Protozoaires et des cellules sexuelles. **11**: 457-648. Arch. Anat. Micros.
- Forenbacher, A.** 1911. Die Chondriosomen als Chromatophorenbildner. Ber. Deutsch. Bot. Ges. **29**: 648-660.
- Garjeanne, A. J. M.** 1903. Die  lk rper der Jungermanniales. Flora **92**: 457-482.
- Goldschmidt, R.** 1904. Der Chromidialapparat lebhaft funktionierender Gewebezellen. Biol. Centralbl. **24**: 241-251.
- Guilliermond, A.** 1911. Sur les mitochondries des cellules v g tales. Compt. Rend. Acad. Sci. (Paris) **153**: 199-201.
- . 1913. Sur les mitochondries des Champignons. Compt. Rend. Soc. Biol. **74**: 618-621.
- . 1914.  tat actuel de la question de l' volution et du r le physiologique des mitochondries. Rev. G n. Bot. **26**: 129-149; 183-208.
- Hertwig, R.** 1899. Ueber Encystierung und Kernvermehrung bei *Arcella vulgaris*. Festschrift f r Kupffer, pp. 367-382. Jena.
- K ster, v.** 1894. Die  lk rper der Lebermoose. Basel.
- Lewis, M. R., and Lewis, W. H.** 1915. Mitochondria (and other cytoplasmic structures) in tissue cultures. Amer. Journ. Anat. **17**: 339-401.
- Lewis, M. R., and Robertson, W. R. B.** 1916. The mitochondria and other structures observed by the tissue culture method. Biol. Bull. **30**: 99-124.
- Lewitsky, G.** 1910.  ber die Chondriosomen in pflanzlichen Zellen. Ber. Deutsch. Bot. Ges. **28**: 538-546.
- . 1911. Die Chloroplastenanlagen, in lebenden und fixierten Zellen von *Elodea canadensis* Rich. Ber. Deutsch. Bot. Ges. **29**: 697-703.
- L wschin, A. M.** 1913. "Myelinformen" und Chondriosomen. Ber. Deutsch. Bot. Ges. **31**: 203-209.
- Lundeg rd, H.** 1910. Ein Beitrag zur Kritik zweier Vererbungshypothesen. Jahrb. Wiss. Bot. **48**: 285-378.
- McAllister, F.** 1914. The pyrenoid of *Anthoceros*. Amer. Journ. Bot. **1**: 79-95.
- Meves, F.** 1904.  ber das Vorkommen von Mitochondrien bzw. Chondromiten in Pflanzenzellen. Ber. Deutsch. Bot. Ges. **22**: 284-286.
- . 1908. Die Chondriosomen als Tr ger erblicher Anlagen, cytologische Studien am H hnerembryo. Arch. mikr. Anat. Ent. **72**: 816-867.
- . 1910.  ber die Beteiligung der Plastochondrien an der Befruchtung des Eies von *Ascaris megaloccephala*. Arch. mikr. Anat. **76**: 683-713.
- Mirande, M.** 1916. Observation sur le vivant de la formation cytologique de l'anthocyanine. Compt. Rend. Acad. Sci. (Paris) **163**: 368-371.
- Mottier, D. M.** 1918. Chondriosomes and the primordia of chloroplasts and leucoplasts. Ann. Bot. **32**: 91-114.

- Nicolosi-Roncati, F. 1912. Genesi dei cromatofori nelle Fucoidee. Bull. Soc. Bot. Ital. 1912, pp. 144-149.
- Pfeffer, W. 1874. Die Oelkörper der Lebermoose. Flora 57: 2-6; 17-27; 33-43.
- Regaud, C. 1911. Les mitochondries, organites du protoplasma considérés comme les agents de la fonction éleclétique et pharmacopexique des cellules. Rev. Méd. 31: agents de la fonction éleclétique et pharmacopexique des cellules.
- Rivett, M. F. 1918. The structure of the cytoplasm in the cells of *Alicularia scalaris*. Annals of Botany 32:
- Rudolph, K. 1912a. Das Chondriom der Pflanzenzelle. Sitzber. "Lotos" Prag 60: 197-199.
- . 1912b. Chondriosomen und Chromatophoren. Ber. Deutsch. Bot. Ges. 30: 605-629.
- Sapêhin, A. A. 1911. Über das Verhalten der Plastiden im sporogenen Gewebe. Ber. Deutsch. Bot. Ges. 29: 491-496.
- . 1913a. Untersuchungen über die Individualität der Plastide. Ber. Deutsch. Bot. Ges. 31: 14-16.
- . 1913b. Ein Beweis der Individualität der Plastide. Ber. Deutsch. Bot. Ges. 31: 321-324.
- Schaudinn, F. 1903. Untersuchungen über die Fortpflanzung einiger Rhizopoden. Arb. Kaiserl. Gesund. 19:
- Scherrer, A. 1913. Die Chromatophoren und Chondriosomen von Anthoceros. Ber. Deutsch. Bot. Ges. 31: 493-500.
- Schmidt, E. W. 1912. Pflanzliche Mitochondrien. Progr. Rei Bot. 4: 163-181.
- Smirnow, A. E. v. 1907. Über die Mitochondrien und den Golgischen Bildungen analoge Strukturen in einigen Zellen von *Hyacinthus orientalis*. Anat. Hefte 32: 143-153.
- Tischler, G. 1906. Über die Entwicklung des Pollens und der Tapetenzellen bei Ribes-Hybriden. Jahrb. Wiss. Bot. 42: 545-578.
- Van der Stricht, O. 1909. La structure de l'oeuf des Mammifères. III^e partie. Mém. Acad. Sci. Belg. II. 2:
- Wakker, J. H. 1888. Studien über die Inhaltskörper der Pflanzenzelle. Jahrb. Wiss. Bot. 19: 423-496.

EXPLANATION OF PLATES XXXIII-XXXIV

All figures were drawn with the aid of the camera lucida, with a Leitz No. 3 ocular and Leitz 1/16 in. oil immersion lens, with the following exceptions: In figure 7 a Leitz No. 6 objective was used; in figures 13 and 14, a Spencer 1/12 in. oil immersion lens.

PLATE XXXIII

- FIG. 1. Cell from plerome of root-tip of corn, showing mitochondria. $\times 900$.
- FIG. 2. Cell from periblem of same preparation, showing mitotic figure and ovoid and spherical mitochondria. $\times 900$.
- FIG. 3. Cell from same region showing similar structures. $\times 1800$. All of above from Regaud's fixation and haematoxylin stain.
- FIG. 4. Cell from plerome of corn root-tip, from plant grown under more favorable conditions. Benda's method of fixation and staining. Vesicular structures show light blue interior. $\times 900$.
- FIG. 5. Two cells from periblem of same preparation. Mitochondria ovoid or spherical. $\times 900$.
- FIG. 6. Series of plastids from same preparation, stained with Flemming's tri-color process. First six in series have red-staining granular inclusions; *g*, *h*, and *i* are stained blue. Iodine test shows starch grains in latter. (FIG. 6 on Plate XXXIV.)
- FIG. 7. Section from young disc of *Preissia*, showing distribution of the granular material in the cells and in the thallus as a whole. $\times 385$.
- FIGS. 8, 9, and 10. Cells taken from same section, showing plastids, etc., on larger scale. $\times 900$.

PLATE XXXIV

FIG. 11. Cell from periphery of young disc of *Preissia*. Benda's method (unbleached). Large bodies with dark borders, violet; smaller, uniformly-colored structures, brown. $\times 1800$.

FIG. 12. Cell from same region, showing only larger bodies. $\times 1800$.

FIG. 13. Portion of older disc, showing development of plastids, with large grains of storage starch. Iodine stain, with Benda's fixation. $\times 800$.

FIG. 14. Two cells from central part of same disc, showing fully developed grains. $\times 800$.

FIG. 15. Series of developing starch grains, from near the periphery of the disc to its center. $\times 900$. (FIG. 15 on Plate XXXIII.)

FIG. 16. Cell from thallus of *Preissia*. Benda's method (unbleached). Large "chloro-leucoplasts" light blue, with dark violet peripheries and filled with starch grains. Smaller bodies, dark brown. $\times 900$.



